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Use of broth enrichment and real-time PCR to exclude the presence of methicillin-resistant *Staphylococcus aureus* in clinical samples: a sensitive screening approach

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ABSTRACT

A rapid and sensitive method for excluding the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical samples was developed and evaluated. The method utilised an MRSA-selective enrichment broth for 16 h, followed by PCR quantification of the *nuc* gene. Samples below a quantitative PCR threshold were reported as MRSA-negative. Broths from PCR-positive samples were subcultured for MRSA isolation. Clinical samples (n = 334) in a constructed high prevalence population were analysed in parallel with a selective plating method. The new broth-PCR assay increased the number of positive samples by 35% (49 vs. 66), and 94% of negative samples were reported within 24 h. To reduce costs and workload, 665 clinical samples were grown separately in enrichment broth and then pooled in the PCR step. The broth-PCR assay increased the number of MRSA positive samples from 11 to 15 compared with selective plating. Most (89%) of the culture-negative samples were also PCR-negative and could be reported within 24 h. The growth of 25 European EMRSA strains was tested in the selective enrichment broth. On average, the MRSA strains showed a 300 000-fold increase in CFU, compared with 30-fold for the eight methicillin-sensitive *Staphylococcus aureus* strains tested.

Keywords Detection, molecular method, MRSA, screening, real-time PCR, selective enrichment

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INTRODUCTION

In most parts of the world, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals has reached levels where its occurrence has to be considered endemic [1]. The associated problems of increased costs and mortality are well-recognised [2–5]. Among the exceptions are the Nordic countries and The Netherlands, where the prevalence of MRSA is <1% among staphylococci causing bacteraemia (EARSS Annual Report 2001; http://www.earss.rivm.nl).

A cornerstone in attempts to prevent an endemic MRSA situation is screening [6]. Patients and staff at risk of being infected or colonised are screened, and ideally kept isolated, until they have been declared free of MRSA. Since some clones of MRSA have a well-documented capacity to spread in the hospital environment [7,8], the surveillance net must be effective. A large proportion of the samples taken will be MRSA-negative. The time between sampling and delivery of the result influences workload and costs for isolation care.

There is an obvious need for a quick, but sensitive, method for identifying MRSA. Every day (or even half-day) saved will reduce costs and workload, and also improve compliance with screening schemes. Several methods have been described that utilise PCR for detection of MRSA directly from clinical specimens [9–11] or in combination with broth enrichment [12,13]. Direct detection enables shorter sample turn-around time in the laboratory, but has the disadvantage of requiring resampling and reanalysis with a method based on cultivation if MRSA isolates are needed for epidemiological analysis. A lower sensitivity and specificity compared with culture methods has also been reported.

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This paper presents an approach that is capable of excluding the presence of MRSA in patient samples in <24 h, with sensitivity superior to the traditional selective plating method used currently in many laboratories. The method is based on selective enrichment in a broth containing methicillin. A quantitative *nuc* gene PCR is then used to exclude the presence of MRSA in the broth, based on the assumption that mainly MRSA, and little else, would be able to multiply in the selective broth. Samples positive in the PCR screen are subcultured for MRSA isolation. The possibility of pooling samples in the PCR step in order to reduce workload and costs was examined, and, as clonal heterogeneity in the level of expression of the methicillin resistance phenotype has been described among MRSA isolates [14,15], the ability of the selective broth to support the growth of 25 European epidemic MRSA and eight MSSA isolates was evaluated.

MATERIALS AND METHODS

Bacterial strains and patient samples

The standard MRSA strains (n = 25) were collected as representative epidemic strains from various member countries in the HARMONY network, and had at least three band differences in *Sma*I pulsed-field gel electrophoresis (PFGE) patterns. PFGE was performed as described by Bannerman *et al.* [16]. The following strains were used: Belgium (BE-1 to BE-3) 97S99 (1432), 97S100 (95/3511/4) and 98S46 (95/1106/2); Finland (FI-1 to FI-6) 61974, 37481, 62396, 75541, 76167 and 54511; France (FR-1 to FR-4) 920, 162, 10828 and 10882; Germany (GE-1 to GE-6) 1442/98, 1450/94, 1155–2/98, 1000/93, 134/93 and 825/96; Greece (GR-1) 3680; Sweden (SW-1) AO9973/97; UK (UK-1 to UK-4) 90/10685, M307, NCTC11939 and 96/32010. Further information on these strains is available on the HARMONY network homepage (http://www.harmony-microbe.net/index.htm).

Seven of the eight MSSA strains used in the study were collected at the Department of Clinical Microbiology, The County Hospital of Halmstad, Sweden. HY54037/00, HY45802/00 and HY54058/00 were isolated from the anterior nostrils of women immediately upon admission to hospital for childbirth. A44138/00, A36655/00, A44130/00 and A100363/99 were isolated from patients with manifest infections during routine diagnostic laboratory work. The strains selected had at least three band differences in the *Sma*I PFGE banding patterns. MSSA strain NCTC 8325 was also included in the test panel.

Between August 2000 and August 2001, 334 MRSA screening samples (77 nostril, 77 pharynx, 77 perineum, 47 wound, 33 urine and 23 from other sites) sent to the laboratory were included in the study. Consecutive samples from known MRSA carriers, together with samples from a presumed negative patient collected on the same day, were included. The 334 screening samples came from 49 different patients (35 MRSA-negative and 14 MRSA-positive on at least one occasion). Nine different MRSA clones, as defined by *Sma*I PFGE patterns, were represented among the 14 positive patients. A further 665 consecutive MRSA screening samples sent to the laboratory between September 2001 and November 2001 were included in the evaluation of pooled samples. Samples were pooled in groups of three consecutive samples.

Selective enrichment broth

The selective enrichment broth (MAM broth) used was based on a solid screening medium for MRSA (MAMSA) described by Perry *et al.* [17]. One litre of MAM broth contains 15 g proteos peptone (Oxoid, Basingstoke, UK), 2.5 g liver digest (Oxoid), 5 g yeast extract (Oxoid), 25 g NaCl, 10 g mannitol, phenyl red 16 mg/L, 2.0 mg methicillin and 8 mg aztreonam (final pH 7.0 ± 0.1).

Growth test of defined strains

Single colonies of *S. aureus* grown on blood agar were transferred to 5 mL of brain-heart infusion broth and grown overnight at 37°C under aerobic conditions. The following day, the culture was vortexed vigorously and an aliquot was diluted serially 10^2, 10^3, 10^4 and 10^5-fold. The inoculum size was determined from the 10^2 dilution using quantitative real-time *nuc* gene PCR (see below), and 50 μL of the dilutions were inoculated into 5 mL of MAM broth. The cultures were incubated in a shaker for 16 h at 37°C under aerobic conditions. To release DNA for PCR analysis, the cultures were harvested by centrifugation, and the bacterial cell pellet was resuspended in 2.5 mL lysis buffer (20 mM Tris-HCl, pH 7.6, Tween 20 (Sigma-Aldrich, Steinheim, Germany) 0.5% v/v, Igepal CA-630 (Sigma-Aldrich) 0.5% v/v and proteinase K (Roche Diagnostics, Indianapolis, IN, USA) 60 mg/L. For cell lysis, the suspension was incubated at 56°C for 1.5 h, followed by proteinase K inactivation at 95°C for 5 min [18].

Clinical samples

All clinical samples were inoculated on to MAMSA plates [17] and incubated at 37°C. The swab was then submerged in a tube containing 5 mL of MAM broth. The MAMSA plate was inspected after 24 and 48 h, and suspected staphylococcal colonies were isolated and analysed further. The broth was incubated for 16 h in a shaker at 37°C under aerobic conditions. Following incubation, a 100-μL aliquot was withdrawn for PCR analysis. The cells were harvested by centrifugation and the resulting pellet was suspended in 50 μL lysis buffer. The suspension was then incubated at 56°C for 1 h, followed by proteinase K inactivation at 95°C for 5 min. In the second part of the study, samples were incubated separately in MAM broth, after which 100-μL aliquots from up to three samples were pooled. Cells were harvested by centrifugation, suspended in 150 μL lysis buffer, and then incubated as described above.

For subculturing of broth cultures, 1 μL was spread on to a blood agar plate without any supplement, a blood agar plate containing lithium chloride 0.5% w/v to suppress the growth of enterococci, and a blood agar plate containing colistin 24 mg/L to suppress the growth of coagulase-negative staphylococci [19].

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These plates were incubated at 37°C overnight and then inspected for growth of staphylococcal colonies. Suspected MRSA isolates were tested by *mecA/nuc* gene PCR (see below).

PCR analysis

For validation purposes, a 10-fold dilution series, with 0.3–3 × 10^6 CFU/PCR reaction, was used. The concentration of bacterial cells was based on viable count determinations on blood agar plates. The suspension of bacteria was prepared for PCR as described for clinical samples, and the serial dilutions were made in the lysis buffer described above, but without addition of proteinase K.

Before PCR analysis, aliquots of lysates were centrifuged for 1 min at 9000 *g*, and 2 μL was subjected to quantitative real-time *nuc* gene PCR. The 20-μL reaction mix consisted of PCR reaction buffer (Roche Diagnostics), DMSO 2% v/v, BSA (Roche Diagnostics) 0.01% w/v, 0.6 × SybrGreen I (Molecular Probes, Leiden, The Netherlands), 0.5 μM each of the oligonucleotide primers (NUC1–5′-GCGATTGATGGTGATACGGTT; NUC3–5′-CAAGCCTTGACGAACTAAAGC) (Scandinavian Gene Synthesis, Köping, Sweden), 4 mM $MgCl_2$, 200 μM dNTPs (Roche Diagnostics), and 0.04 U/μL of FastStart *Taq*Polymerase (Roche Diagnostics). The primers used for *nuc* gene amplification were those described by Brakstad *et al.* [20], with the exception of the NUC3 reverse primer, which was shortened by three nucleotides at the 5′-end, compared with the published sequence. The reaction mix was incubated in a LightCycler (Roche Diagnostics), with preincubation at 95°C for 10 min followed by 35 cycles of 95°C for 15 s, 65°C for 5 s and 72°C for 12 s. Fluorescence values for each capillary were measured at 530 nm at the end of each extension phase. Each experiment was completed by a melting point analysis: 95°C for 0 s (hold time) with 20°C/s transition rate, 60°C for 30 s with 20°C/s transition rate, 70°C for 0 s (hold time) with 20°C/s transition rate, and 90°C for 0 s (hold time) with 0.2°C/s transition rate, with continuous acquisition of fluorescence data at 530 nm.

The number of cells carrying the *nuc* gene in the lysates was determined by comparing the crossing-point of each sample with a standard curve. The standard curve consisted of suspensions of MRSA cells at known concentrations (CFU/mL), as determined by viable counts on blood agar, determined as described above. Three standard concentrations (3.2 × 10^4, 3.2 × 10^6 and 3.2 × 10^8 CFU/mL) were assayed in each LightCycler experiment, and the resulting crossing-point values were used to generate the standard curve.

Verification of MRSA identification was done by amplification of the *nuc* gene and the *mecA* gene. One colony was suspended in 50 μL lysis buffer and lysed, followed by amplification of the *nuc* gene as described above. The *mecA* gene was amplified using the same protocol, but using primers MEC3 (5′-GCAATCGCTAAAGAACTAAG) and MEC4 (5′-GGGACCAACATAACCTAATA) (Scandinavian Gene Synthesis) to amplify a 222-bp fragment of the *mecA* gene. This fragment had a melting peak at 81.6°C (average of 15 separate runs of control strain; range 81.2–82.1°C; standard deviation 0.23°C) in the melting point analysis.

Control for PCR inhibition

In total, 100 consecutive sample pools (240 samples) were tested for possible inhibition. A bacterial lysate, prepared as described above from two or three pooled samples, was seeded with a diluted lysate from a positive MRSA strain (CCUG 35600). The seeded lysate contained MRSA DNA, corresponding to *c.* 10^5 CFU/mL. As a reference sample, lysis buffer without bacteria was seeded with the same amount of positive material. The samples were analysed in the LightCycler as described above, and crossing-points were calculated. Using this approach, inhibition in a sample is seen as a higher crossing-point value than for the reference sample.

RESULTS

Validation of *nuc* gene PCR

The specificity of the *nuc* gene PCR [20] in the SybrGreen format was established by analysing the size of the amplicons generated with a positive control strain (MRSA CCUG 35600). Amplification produced a DNA fragment of the expected size (276 bp), as determined by gel electrophoresis, which corresponded to a melting peak at 82.6°C (average of 50 separate runs of the control strain; range 81.6–84.2°C; standard deviation 0.44°C). A peak of lower melting temperature was also observed in positive samples, but not in negative samples (Fig. 1) or the negative control, as would be expected if it were the result of primer-dimer amplification.

The detection level, PCR efficiency and linearity of the *nuc* gene PCR was investigated by analysing dilutions of MRSA CCUG 35600 containing known numbers of CFU. Fig. 2 shows the

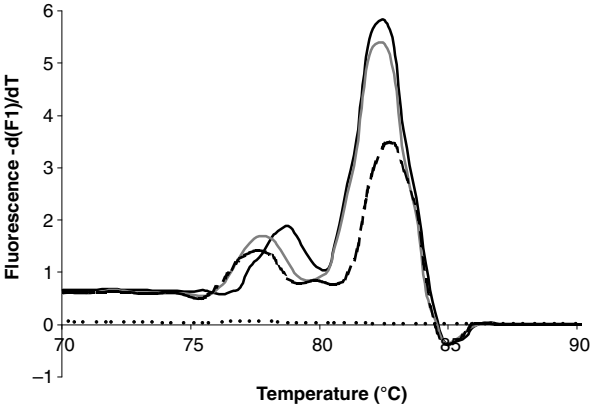

Fig. 1. Melting point analysis of *nuc* gene amplification reaction with MRSA control strain and patient samples as template. Melting peak at 82.6°C corresponds to the 276-bp *nuc* amplicon. Black line, standard MRSA CCUG 35600 (viable count of 1.6 × 10^9 CFU/mL); grey line, patient sample quantified to 1.3 × 10^7 CFU/mL; broken black line, patient sample quantified to 1.0 × 10^3 CFU/mL; dotted line, negative patient sample.

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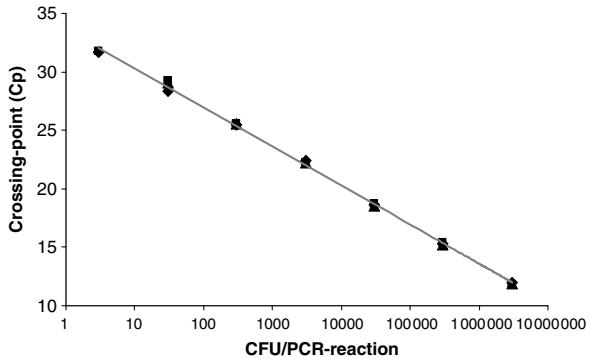

Fig. 2. Linearity of the *nuc* gene amplification. Triplicates of dilutions ranging from 0.3 to 3×10^6 CFU/reaction were amplified. No amplification occurred with 0.3 CFU/reaction. Two of three triplicates were positive with 3 CFU/reaction. Regression line shown is calculated from the average of triplicates (duplicate for 3 CFU dilution) for the different dilutions. $R^2 = 0.9999$.

crossing-point values plotted against triplicate values of dilutions ranging from 3 to 3×10^6 CFU/PCR. All triplicates down to 30 CFU/reaction generated amplification curves, and two of three were positive with 3 CFU/reaction. No amplification occurred with the dilution containing 0.3 CFU. The reaction was linear over at least five orders of magnitude, with an R^2 value of 0.9999, based on the average crossing-point value for triplicates containing 30–3×10^6 CFU/reaction. The slope of the regression line was −3.41, corresponding to a PCR efficiency of 96%.

In order to validate the quantitative real-time *nuc* gene PCR, a separately prepared MRSA control containing 6.7×10^5 CFU/mL was prepared. This standard was quantified in ten separate runs from a three-point standard curve. The mean number of cells in the standard was 5.57×10^5 CFU/mL (range, 4.5×10^5–7.1×10^5 CFU/mL; standard deviation, 7.98×10^4; coefficient of variation, 14.3%). The accuracy was −16.4% (5.6×10^5–6.7×10^5/6.7×10^5 = −0.164).

Evaluation of MAM broth with defined strains

The growth of 25 standard epidemic MRSA strains and eight clinical isolates of MSSA was tested as described in Methods. The initial inoculum for the 25 MRSA strains varied between 200 and 1900 CFU/mL (mean 908 CFU/mL), while that of the eight MSSA isolates was between 450 and 9850 CFU/mL (mean 2550 CFU/mL). Fig. 3 shows the increase in cell-count for each strain after incubation for 16 h. On average, the 25 MRSA strains showed a 3×10^5-fold increase (range 4000–1.7×10^6), compared with 30-fold (range 0–130-fold) for the eight MSSA isolates. All MRSA strains tested were able to multiply in the broth to a greater extent than the tested MSSA isolates.

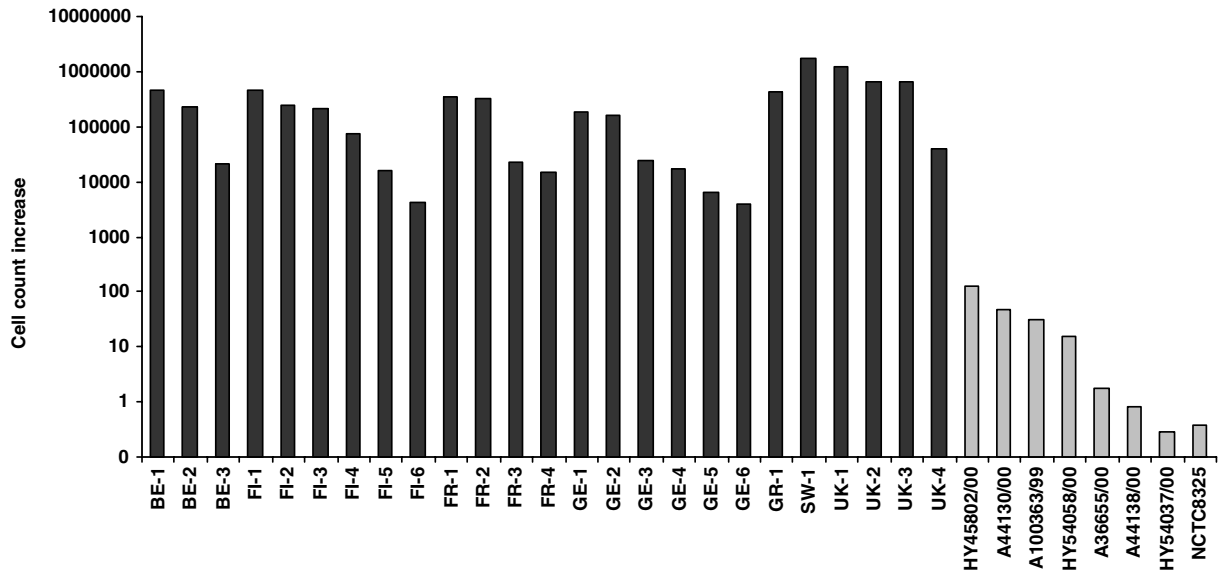

Fig. 3. Fold-increase in cell counts for 25 MRSA and eight MSSA following incubation for 16 h in MAM broth. Black bars, MRSA; grey bars, MSSA.

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Analysing clinical samples

The enrichment-PCR method described here was compared with a sensitive selective plating method [17]. In order to evaluate the sensitivity within a reasonable time, a series with a high prevalence (20%) of MRSA-positive samples was created. Sample series from known MRSA-positive individuals were included consecutively in the study, together with an additional sample series from a presumed MRSA-negative individual. Between August 2000 and August 2001, 334 clinical samples were analysed with both methods. In total, MRSA was isolated from 67 of the 334 samples with either of the methods; 66 after enrichment followed by subculturing, and 49 following inoculation on to a MAMSA plate. The broth failed to detect one positive sample (Table 1).

With the aim of using the *nuc* gene PCR as a screening tool, a cut-off for MRSA negativity was defined. As shown in Fig. 4, all samples from which MRSA was isolated contained $>1 \times 10^5$ CFU/mL. It was concluded that samples containing $<5 \times 10^4$ CFU/mL could safely be considered negative for MRSA. Using the value of 5×10^4 CFU/mL as the cut-off for positivity, the PCR step identified all MRSA-containing broth-cultures with a specificity of 94% (Table 1; Fig. 4). Thus, 94% of the negative samples, in this constructed 20% prevalence population, were reported within 24 h. The remaining negative and positive samples required an additional 24 h before becoming conclusive. Seventeen samples which, according to the PCR, contained $>5 \times 10^4$ CFU/mL, failed to yield MRSA colonies on the MAMSA plate or from the MAM broth. In most of these cases, MSSA colonies were obtained after subculturing the broth.

Table 1. Comparison of the performance of the selective MAMSA plate (MAMSA), the real-time *nuc* gene PCR and the selective MAM broth (BROTH) for isolation of MRSA

	MAMSA	PCR	BROTH
True-positive	49	66	66
True-negative	267	250	267
False-positive	0	17	0
False-negative	18	1	1

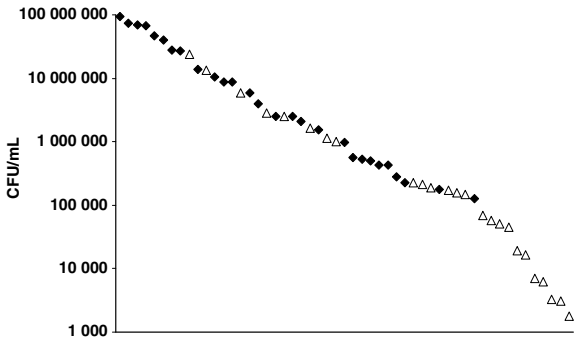

Fig. 4. Plot of *Staphylococcus aureus* counts (CFU/mL) determined by quantitative real-time PCR for a subset of 334 clinical samples (53/334) around the cut-off value of 50 000 CFU/mL. The remainder of the samples were either MRSA-positive with $>1 \times 10^9$ CFU/mL (38/334 samples) or MRSA-negative and below the 1000 CFU/mL detection level of the method (243/334 samples). Black rectangles, MRSA-positive samples; white triangles, MRSA-negative samples.

The frequency of MRSA-positive results from the nostril, pharynx and perineum screening sites increased by 76% (from 17 to 30 positives) following use of an enrichment broth. In contrast, the rate only increased by 13% (from 32 to 36 positives) for sites at which infection rather than colonisation was expected (Table 2).

These results were obtained by performing one PCR for each clinical sample. In order to reduce the workload and cost for the laboratory, the possibility of pooling samples for the PCR step was investigated. All swabs (n = 665) were first inoculated on to a MAMSA plate and then immersed in selective broth. Broth cultures from three consecutive samples were pooled in the PCR step and then analysed by quantitative *nuc* gene PCR, although only those samples that were positive above the threshold were subcultured on to agar plates for MRSA isolation. Based on the threshold defined previously, and the assumption that a single positive sample in a pool with two negative samples would be diluted three-fold, a threshold of $5.0 \times 10^4 \div 3 = 1.7 \times 10^4$ CFU/mL was used. Of 665 samples analysed, MRSA was isolated from 15, leaving 650 negative samples. Of these, 579 (89%) were below the cut-off point in the PCR analysis. All 15 positive samples were positive by PCR, and MRSA was isolated from the

Table 2. Body sites from which MRSA was isolated following direct plating on a MAMSA plate and following enrichment in selective broth (total of 334 clinical samples)

	Nostril	Pharynx	Perineum	Wound	Urine	Other
Plate	3	7	7	15	8	9
Broth	6	16	8	16	8	12

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broth, while 11 of 15 were positive on the MAMSA plate.

Inhibition test

Consecutive pools ($n = 100$) of samples were tested for inhibition following seeding with small amounts of positive DNA lysate. On average, the crossing-point value for seeded pools was 1.50 cycles higher than that for the reference sample. The deviation from the reference sample was within the range 0.36–3.86, with a standard deviation of 0.78 (as a reference value, a ten-fold dilution of a sample is expected to increase the crossing-point value by *c*. 3.3, assuming a PCR efficiency of 100%).

DISCUSSION

The method described in this paper has been in regular use in this laboratory since November 2001, and the basic idea has also been adopted in other clinical laboratories in Sweden [13]. The method is based on the assumption that *nuc*-carrying bacterial cells will proliferate in the selective broth to a level above the defined cut-off when a *mecA* gene is present in the same cell (MRSA). Initially, *nuc*-positive broth samples were assayed for the presence of *mecA* genes, but this had limited value (results not shown), probably because of a high frequency of *mecA* genes in coagulase-negative staphylococci present in the samples.

Many different solid culture media [17,21,22] and enrichment broth media [12,23–26] have been described for the cultivation of *S. aureus*. Some media include NaCl at high concentrations to favour growth of staphylococci, although some clones have a documented lower salt-tolerance [27], as well as oxacillin or methicillin to make them selective for MRSA. The new methicillin, aztreonam and mannitol-containing broth medium described in the present study was based on MAMSA agar [17], with a relatively low NaCl concentration. It was designed to selectively enrich for MRSA after incubation overnight (for 16 h).

It is well-known that different strains of MRSA express the methicillin-resistant phenotype at different levels [14,15,28]. This largely unexplained property must be taken into account when designing selective media for MRSA, and evaluations should include appropriate relevant strains. The present study used part of the HARMONY collection of epidemic MRSA strains, and demonstrated that all tested strains ($n = 25$) were able to multiply in the enrichment broth described above. Although the inoculum sizes varied for the different strains, no correlation was observed between inoculum size and the increase in cell counts (results not shown). The main conclusion was that all tested MRSA strains multiplied to a greater extent than all tested MSSA strains, even though the inoculum of the MSSA strains was, on average, larger than that of the MRSA strains. The performance of the broth was not tested with very small numbers of MRSA cells, but its good performance, especially with throat samples, indicated a capacity to support the growth of MRSA from clinical samples containing low numbers of MRSA.

The relatively low selective pressure in the broth allows some strains of MSSA to grow, which will lead to false-positive PCR results, as was the case for 6% of the 267 negatives when analysing single samples, and 11% of the pooled samples. MSSA was isolated from most of these samples after subculture of the broth. Experience indicates that it is necessary to keep the methicillin concentration at a moderate level in order to allow low-grade (or heterogeneously) resistant MRSA clones to grow. It may be possible to increase the specificity by using other selective substances, e.g., ceftizoxime [26]. A sample containing a large ($>5 \times 10^5$ CFU/mL) inoculum of MSSA could also produce a false-positive PCR result from non-viable cells, even without growth of MSSA in the broth. MRSA was isolated after direct plating of one of 67 positive samples on the MAMSA plate, but not after broth enrichment. The *nuc* gene PCR was also negative for this sample. Since only a few colonies of MRSA were seen on the MAMSA plate, the discrepancy might be caused by the fact that the plate was inoculated before the broth.

By pooling the samples in the PCR step, the number of PCRs was reduced substantially, and consequently the 'hands on' time and the volume of PCR reagents were also reduced. Since only 32 samples can be analysed simultaneously in the LightCycler, pooling also reduces machine usage substantially. During evaluation, samples were pooled consecutively, thereby grouping samples from different patients in the same pool. Samples

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from MRSA-negative patients will be delayed if pooled with samples from a positive patient. This delay could be avoided simply by not mixing samples from different patients.

In total, 100 sample pools (240 samples) were tested for the presence of PCR inhibitors. Total inhibition was not observed, and it was concluded that it would not be cost-effective to introduce an inhibition control for all samples. This is in agreement with the findings of Fang *et al.* [13], who observed inhibition in only one of 260 samples tested. The broth-PCR method increased the detection rate substantially for MRSA in samples from nostrils and throat, but only slightly for samples from other sites. This may be caused by low-level colonisation or carriage of MRSA at these sites, which is below the detection level of many conventional methods. This could explain, in part, why individuals colonised or infected with MRSA for longer periods can test negative for weeks, and sometimes months, and then appear to be re-colonised with the same clone.

Overall, the MRSA screening method described in this report has several advantages. The ability to exclude the presence of MRSA within 24 h will reduce costs and workload, and also improve compliance with screening schemes. Improved sensitivity will result in fewer undetected MRSA-positive patients and staff in the healthcare system.

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REFERENCES

1. Diekema DJ, Pfaller MA, Schmitz FJ *et al.* Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–99. *Clin Infect Dis* 2001; **32** (suppl 2): S114–132.
2. Ibelings MM, Bruining HA. Methicillin-resistant *Staphylococcus aureus*: acquisition and risk of death in patients in the intensive care unit. *Eur J Surg* 1998; **164**: 411–418.
3. Romero-Vivas J, Rubio M, Fernandez C, Picazo JJ. Mortality associated with nosocomial bacteremia due to methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 1995; **21**: 1417–1423.
4. Topeli A, Unal S, Akalin HE. Risk factors influencing clinical outcome in *Staphylococcus aureus* bacteraemia in a Turkish University Hospital. *Int J Antimicrob Agents* 2000; **14**: 57–63.
5. Whitby M, McLaws ML, Berry G. Risk of death from methicillin-resistant *Staphylococcus aureus* bacteraemia: a meta-analysis. *Med J Aust* 2001; **175**: 264–267.
6. Verhoef J, Beaujean D, Blok H *et al.* A Dutch approach to methicillin-resistant *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 461–466.
7. Oliveira DC, Tomasz A, de Lencastre H. Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant *Staphylococcus aureus*. *Lancet Infect Dis* 2002; **2**: 180–189.
8. da Silva Coimbra MV, Silva-Carvalho MC, Wisplinghoff H *et al.* Clonal spread of methicillin-resistant *Staphylococcus aureus* in a large geographic area of the United States. *J Hosp Infect* 2003; **53**: 103–110.
9. Warren DK, Liao RS, Merz LR, Eveland M, Dunne WM. Detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swab specimens by a real-time PCR assay. *J Clin Microbiol* 2004; **42**: 5578–5581.
10. Francois P, Pittet D, Bento M *et al.* Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. *J Clin Microbiol* 2003; **41**: 254–260.
11. Huletsky A, Giroux R, Rossbach V *et al.* New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J Clin Microbiol* 2004; **42**: 1875–1884.
12. Jonas D, Speck M, Daschner FD, Grundmann H. Rapid PCR-based identification of methicillin-resistant *Staphylococcus aureus* from screening swabs. *J Clin Microbiol* 2002; **40**: 1821–1823.
13. Fang H, Hedin G. Rapid screening and identification of methicillin-resistant *Staphylococcus aureus* from clinical samples by selective-broth and real-time PCR assay. *J Clin Microbiol* 2003; **41**: 2894–2899.
14. Matthews PR, Stewart PR. Resistance heterogeneity in methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiol Lett* 1984; **22**: 161–166.
15. Hartman BJ, Tomasz A. Expression of methicillin resistance in heterogeneous strains of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1986; **29**: 85–92.
16. Bannerman TL, Hancock GA, Tenover FC, Miller JM. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *J Clin Microbiol* 1995; **33**: 551–555.
17. Perry PL, Coombs GW, Boehm JD, Pearman JW. A rapid (20 h) solid screening medium for detecting methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 1998; **40**: 67–72.
18. Brakstad OG, Maeland JA, Tveten Y. Multiplex polymerase chain reaction for detection of genes for *Staphylococcus aureus* thermonuclease and methicillin resistance and correlation with oxacillin resistance. *APMIS* 1993; **101**: 681–688.
19. Heltberg O, Bruun B. Polymyxin susceptibility in staphylococci differentiating coagulase-positive and coagulase-negative strains. *Acta Pathol Microbiol Immunol Scand (B)* 1983; **91**: 157–161.
20. Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J Clin Microbiol* 1992; **30**: 1654–1660.

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21. Lally RT, Ederer MN, Woolfrey BF. Evaluation of mannitol salt agar with oxacillin as a screening medium for methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 1985; **22**: 501–504.
22. Baird-Parker AC. An improved diagnostic and selective medium for isolating coagulase positive staphylococci. *J Appl Bacteriol* 1962; **25**: 12–19.
23. Ford M, Perry JD, Robson I *et al*. Evaluation of tube coagulase and a fluorogenic substrate for rapid detection of methicillin-resistant *Staphylococcus aureus* from selective enrichment broth in an outbreak of EMRSA 15. *J Hosp Infect* 1999; **41**: 133–135.
24. Cookson BD, Webster M, Phillips I. Control of epidemic methicillin-resistant *Staphylococcus aureus*. *Lancet* 1987; **1**: 696.
25. Sautter RL, Brown WJ, Mattman LH. The use of a selective staphylococcal broth v direct plating for the recovery of *Staphylococcus aureus*. *Infect Control Hosp Epidemiol* 1988; **9**: 204–205.
26. Wertheim H, Verbrugh HA, van Pelt C, de Man P, van Belkum A, Vos MC. Improved detection of methicillin-resistant *Staphylococcus aureus* using phenyl mannitol broth containing aztreonam and ceftizoxime. *J Clin Microbiol* 2001; **39**: 2660–2662.
27. Jones EM, Bowker KE, Cooke R, Marshall RJ, Reeves DS, MacGowan AP. Salt tolerance of EMRSA-16 and its effect on the sensitivity of screening cultures. *J Hosp Infect* 1997; **35**: 59–62.
28. Chambers HF. Methicillin resistance in staphylococci. molecular and biochemical basis and clinical implications. *Clin Microbiol Rev* 1997; **10**: 781–791.

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